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Insight on trans-plasma membrane behavior of virus-infected plant cells

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Abstract: Little is known about the ion fluxes generated during plant-virus interactions, despite significant losses caused by viruses to agricultural crops. Changes in average ion currents were identifying an early event in the signal transduction pathway related to virus/host interaction. While significant decrease in the average inward currents, mainly due to Ca^{2+} moving into the cell was observed, the role of potassium may be significant. Host specific K^+ efflux with a concomitant decrease in the intracellular K^+ was observed in tobacco plants during the early minutes after infection, suggesting many hypothesis about the role of potassium in host-virus interaction. In the last years, trans-plasma membrane potential was evaluated for some viruses, observing as effect on membrane was different in relation to virus infection and host. Conversely, settle virus infection generally lead to an increase of activity in trans-plasma membrane electron transport.

1. Introduction

In animal cells, the role of plasma membrane behavior is deeply investigated, and it may reflect health status. Cellular defense against stress is a key function in which the plasma membrane redox system is involved. Moreover, some of the plasma membrane redox system enzymes produce reactive oxygen species as well as play a protective role against them. Moreover, evidences underline as redox state of the cell and, accordingly, plasma membrane electron transport contribute to control cell growth, development and apoptosis (Ly and Lawen, 2003). In animal cells, virus infection can lead to variation in membrane potential (Akeson *et al.*, 1992). These effects may occur even during early pathogen recognition events in plant-microbe interaction. As reviewed by Elmore and Coaker (2011), plasma membrane H^+-ATPases are dynamically regulated during plant immune responses and quantitative proteomics studies suggest complex spatial and temporal modulation of plasma membrane H^+-ATPase activity during early pathogen recognition events, even if no data relative to virus were available. Anyway, an important role is played by H^+-ATPases in bacterial infection, where the enzyme cooperate with plant immune signaling protein (namely RIN4) to regulate stomatal apertures during pest invasion of leaf tissue. These enzymes are ubiquitous and are involved in plant immune responses. Moreover, they may are targeted by pathogens to increase plant susceptibility, even if these evidences are related to fungi or bacteria. Thus Shabala *et al.* (2010) affirm that little is known about the ion fluxes generated during plant-virus interactions, despite significant losses caused by viruses to agricultural crops. Viruses represent a major threat to agricultural production, mainly due to the lack of effectiveness treatments, and preventative measures represent the main tools available to protect plants. While it is widely believed that in many seed-propagated crops virus the threat is of limited importance, the health status of plants subjected to vegetative propagation is critical. Although the viruses that infect woody plants raised more

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health concerns, trials are longer and more difficult to manage compared to test with herbaceous plants.

2. Viruses and hosts: a poorly investigated binomial in trans-plasma membrane events

Few study investigated the trans-plasma membrane behavior of virus-infected plants, thus few viruses were evaluated in trials, mainly focused on herbaceous plant-related virus. *Tobacco mosaic virus* (TMV) is a widespread virus difficult to eradicate or control from plant and soils (Luvisi *et al*., 2012 a; Panattoni *et al*., 2013 a; Luvisi *et al*., 2015 a). TMV was frequently chosen as "model virus" in host-virus interaction tests. It was also chosen in first studies in trans-plasma membrane behavior of plants (Schvarzstein, 1997). *Cucumber mosaic virus* (CMV) (Rinaldelli *et al*., 2012), *Papaya mosaic virus* (PMV) (Schvarzstein, 1997), *Potato virus X* (PVX) (Shabala *et al.*, 2010) and *Tobacco ringspot virus* (TRSV) (Stack and Tattar, 1978) were also investigated.

Among virus affecting woody plants, grapevine viruses (Rizzo *et al.*, 2012, 2015) were the most investigated. *Grapevine leafroll associated virus* 1 or 3 (GLRaV-1, GLRaV-3), *Grapevine fanleaf nepovirus* (GFLV), *Grapevine fleck maculavirus* (GFkV), *Grapevine vitivirus A* (GVA) and *Arabis mosaic virus* (ArMV) were evaluated during antiviral tests or in settle infections (Panattoni *et al.*, 2013 b; Rinaldelli *et al.*, 2014).

3. Trans-plasma membrane behavior in early virus infection events

Few data are available for early events in plant-virus interaction. Stack and Tattar (1978) observed as TRSV-infected cells of cowpea showed altered trans-membrane electropotentials and responded differently from healthy cells when subjected to the metabolic inhibitor sodium azide in their ability to recover. Changes in average ion currents were also observed in protoplast of *Gomphrena globosa* artificially infected by PMV and TMV, identifying an early event in the signal transduction pathway related to virus/host interaction (Schvarzstein, 1997). In *G. globosa*, necrotic lesions surrounded by a chlorotic ring developed in inoculated leaves are thought to be the results of a non-host hypersensitive response (HR) that ultimately defend the plants from virus infection. Schvarzstein (1997), using patch-clamp techniques, elucidated the early events occurring during PMV infection. Whole cell recording indicate a significant decrease in the average inward currents, mainly due to Ca^{2+} moving into the cell or gluconate moving out of the cell. In the same time, an increase in the outward currents was also observed, due to Cl^- moving into the cell and K^+ moving out of the cell. The outward currents were correlated to PMV concentration, and a similar behavior was observed during TMV infection. The studies of Schvarzstein were the first one in which ion fluxes were characterized during virus-plant interaction and unfortunately, very few studies following.

The phenomenon of rapid alteration of ion fluxes in host cells during the early phase of viral infection was reported in *Chlorella* (Neupartl *et al*., 2008). *Chlorella* viruses tests suggested a key-role of potassium, due to an observed host specific K^+ efflux with a concomitant decrease in the intracellular K^+. The use of blockers of the viral-encoded K(+) channel (Kcv) reduced this K^+ efflux, suggesting as depolarization and K^+ efflux are at least partially mediated by Kcv. The K^+ efflux seems virus-triggered and it occurs in the same time frame as host cell wall degradation and ejection of viral DNA. Therefore, Neupartl *et al.* (2008) supposed that loss of K^+ and associated water fluxes from the host lower the pressure barrier to aid ejection of DNA from the virus particles into the host. Even if *Chlorella* shared some metabolic pathway with plants, this interesting findings cannot directly transferred to plants without further studies. Moreover, DNA-based viruses are uncommon pathogens in plants. Anyway, the key-role of potassium in plant-virus interaction was confirmed by Shabala *et al.* (2010), moving the focus from calcium. The role of ion fluxes in plant defense signaling is well documented and elicitor-induced transient Ca^{2+} influx from the external environment into the cytosol has always been named as a key element of the signaling cascade and appears to be crucial in the induction of plant defense against pathogens (Scheel, 1998; Zimmermann *et al*., 1999; Blume *et al*., 2000; Grant *et al.*, 2000), but this studies are not related to viruses. Shabala *et al*. (2010) observed as an addition of the purified PVX to the mesophyll tissue of tobacco plants caused no changes in the rate of Ca^{2+} transport across the plasma membrane. These evidences were supported by no significant changes in concentration of Ca^{2+} in cytosol for at least 50 min after PVX treatment. This behavior indicated that Ca^{2+} release

from internal stores was also not a part of the signal transduction mechanism in plant-viral interaction. Authors suggest that, contrary to bacterial pathogens, rapid Ca^{2+} signaling may not be essential for the viral perception and initiation of downstream transduction pathway. Conversely, massive K^+ efflux was measured as early as 10 min after virus inoculation and this behavior in host-related. Prolonged exposure virus caused lower concentration of Ca^{2+} in cytosol compared with control plants, suggesting of the role of Ca^{2+} efflux systems in downstream cascades of the plant responses to viral infection. K^+ efflux was partially reduced by blockers of Kcv channels such as Cs^+ (Shabala *et al*., 2010), similarly to *Chlorella* tests. The antiviral drug mycophenolic acid (MPA) (Panattoni *et al.*, 2014; Guazzelli *et al*., 2015) was also indicated as interferent with K_{ATP} channel activity in grapevine, as well as inhibiting activity of the inward-rectifier potassium ion channel which could be mediated by guanosine depletion induced by MPA (Luvisi *et al.*, 2015 b).

Nowadays, the physiological role of the observed viral-induced K^+ efflux was not known. Neupartl *et al*. (2008) suggest that the efflux of K^+ and the associated water efflux from the *Chlorella* cell may be needed to reduce turgor and lower the pressure barrier, to aid ejection of DNA from the virus particles into the host, but adding several known blockers of K^+ efflux channels to the buffer media during PVX inoculation in tobacco plants did not ameliorate infection symptoms, probably because the blocking effect of pharmacological agents was only partial (Shabala *et al.*, 2010). Further hypothesis may be related to role of potassium homeostasis in plant adaptive responses to environment (Shabala *et al.*, 2007), including programmed cell death (Shabala, 2009).

Interestingly, the observed viral-induced activation of K^+ efflux systems appears to be a highly host-specific process, indicating that the observed K^+ fluxes may be linked to the plant's ability to recognize compatible viral infection and activate defense pathways (Shabala *et al*., 2010).

4. Trans-plasma membrane behavior in settle virus infection

Membrane potential

Membrane potential of grapevine cells were monitored during antiviral drugs treatments (Luvisi *et al.*, 2012 b). The complex of results obtained in this research highlights, first of all, that membrane electrical response of the tested antiviral drugs is supported by the metabolism of plant cell, and no differences in Δ Em were found GLRaV-1-infected grapevine leaves compared to virus-free leaves. However the behavior of membrane of plants treated with antiviral drugs was different in other hosts, as reported in tobacco plants infected by CMV, where trans-plasma membrane potential activity seemed to be influenced by virus presence, acting differently in infected or healthy samples during drug uptake by cells (Rinaldelli *et al*., 2012).

Anyway, drugs may hindered or enhance virus effects of membrane behavior and specific trials in settle virus infection were carried out in grapevine (Rinaldelli *et al*., 2014). Samples infected by leafroll viruses showed no difference in membrane potential values compared to healthy samples, confirming the similar behavior of GLRaV-1 or -3 infected samples and healthy ones observed during antiviral drug treatments. GFLV, or ArMV infected tissues led to plasma membrane hyperpolarization with higher values compared to healthy samples, while GFkV and GVA infected tissues showed plasma membrane depolarization significantly lower than the control (Rinaldelli *et al*., 2014). According to the role of H^+-ATPase activity in generating trans-plasma membrane potential gradient (Sondergaard *et al*., 2004), GFLV and ArMV infected cells could be considered more energized compared to others. Conversely, GFkV and GVA infection was also associated to increasing difficulty of cell membrane measurements, as occurs under stress conditions (Rawyler *et al*., 2002).

Electron transport

Rubinstein and Luster (1993) indicated that that trans-plasma membrane electron transport (t-PMET) occurs in all types of organisms, including plants. t-PMET allows reduction of extracellular oxidants at the expense of intracellular reducing equivalents that may derive from NADH or NADPH (Del Principe *et al*., 2011). The t-PMET enzymes have been investigated based on their ability to reduce external artificial impermeant electron acceptors. Ferricyanide (Fe^{3+}) has been commonly used as electron acceptor in assays performed with intact cells; ferricyanide is converted to ferrocyanide (Fe^{2+}), and the rate of this reduction can be monitored. Using a carbon fibre microelectrode (CFME) it is possible to map oxidoreductase activity using impermeant electron acceptors or donors (Taylor and Chow, 2001). This techniques

were also used to evaluate *in vivo* estimation of the inosine monophosphate dehydrogenase inhibition caused by antiviral drugs (Panattoni *et al.*, 2015).

The t-PMET was observed during antiviral treatments in CMV-infected tobacco plants (Rinaldelli *et al*., 2012). Infected samples were less sensitive to antiviral treatments considering t-PMET. This effect may be due to the concurrent entry of drug within the symplast that, as indicated by membrane potential, was lower in infected samples and that can lead to lower inhibition of NAD^+/NADH conversion by drug and to the following increase of Fe^{3+} conversion.

This virus-related behavior was confirmed in GLRaV-1 and -3 tests (Panattoni *et al.*, 2013 b). Virus-infected samples exhibited elevated t-PMET activity compared to healthy samples. The [Fe^{2+}] in healthy samples was set at 26 µM, while GLRaV-1- and GLRaV-3-infected samples showed 34-49 µM [Fe^{2+}]. These data can be linked to the NADH content in GLRaV-1 and GLRaV-3 samples, that was set at 1.2 times higher than healthy samples. This trans-plasma membrane behavior was partially confirmed for other grapevine viruses (Rinaldelli *et al.*, 2014). In virus-infected samples, while the [Fe^{2+}] produced by GFkV and GVA infected tissues were similar to healthy tissues, the samples infected by GFLV, ArMV and leafroll viruses showed higher t-PMET activity. The higher NADH content due to virus infection was used differently by infected samples during their t-PMET activity. Samples whose infectious status did not interfere negatively with membrane potential, such as ArMV, GFLV, GLRaV-1, and -3 showed higher t-PMET activity compared to healthy samples, in agreement with the higher NADH availability.

5. Discussion

The high level of specialization attained by many viruses due to their replication and pathogenetic mechanisms towards the host make them an extremely variable and complex target. This complexity is the background upon which a defensive strategy can be optimized. Therefore, the outcome of therapeutic action is strongly influenced by the ontological properties of the virus to be eliminated as well as the characteristics expressed by the plant as well as the trans-membrane transport of drugs. In human pathology, trans-membrane alteration may represent signalling systems for regulating cellular metabolism able to interfere with distinct cellular functions such as redox homeostasis and pathogens defense (Herst and Berridge, 2006), but few data are available in plants. For systemic viruses such as TMV, movement of viral particles occurs by infected cell to nearby healthy ones, and subsequent systemic transport is governed by a series of mechanisms involving various virus and plant factors (Scholthof, 2005), interacting with the host cell membrane, binding to some cytoskeletal proteins which could include changes in ion fluxes and signals in the transduction pathway (Atkinson *et al.*, 1996), thus further studies in trans-plasma membrane behavior of virus-infected cells are desirable

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